Chapter 2

REVIEW OF LITERATURE
2.1 HISTORY OF MUCOPOLYSACCHARIDOSES

The first description of mucopolysaccharidoses probably was in the early 20th century as cited (Dorfman and Matalon, 1976). Later a clear description of two brothers who appeared to suffer from mucopolysaccharidosis, which is now referred as MPS II or Hunter syndrome was presented (Hunter 1917). Two years later two unrelated boys with coarse facial features, multiple skeletal abnormalities, corneal clouding, hepatosplenomegaly and cardiac involvement were described (Hurler, 1919). This detailed description was presumed to be Hurler syndrome or MPS I and served as the prototype for all MPS that followed. Earlier this group of disorders were collectively referred by the misnomer 'gargoylism' because of the supposed resemblance of the patients to the gargoyles of certain cathedrals. In the mid 20th century, the genetic nature of this disease was recognized and two types of gargoylism were suggested (Wolf, 1942). One type with an autosomal recessive mode of inheritance (Nja, 1946) later known as Hurler syndrome and the other with X-linked recessive inheritance, now known as the Hunter syndrome.

However, the term mucopolysaccharidoses was introduced based on the findings of excessive mucopolysaccharide accumulation in the liver of the patients with gargoylism (Brante, 1952). This was followed by the identification of the mucopolysaccharides, heparan sulfate and dermatan sulfate in the urine of these patients and provided the basis for a routine biochemical diagnostic test for these conditions (Dorfman and Lorincz, 1957). During the 60s further delineation of MPS resulted following the clinical
description of Scheie (Scheie et al., 1962) Sanfilippo (Sanfilippo et al., 1963) and Maroteaux - Lamy (Maroteaux et al., 1963) syndromes. These patients also excreted excessive mucopolysaccharides (glycosaminoglycans - GAGs) in their urine which was misinterpreted as due to the exaggerated synthesis of GAGs. Later based on electron microscopic studies (Van Hoof and Hers, 1964) the GAG accumulation was shown to be due to a defect in the degradation and not synthesis of GAGs.

Intense interest in the study of MPS began in 1960s, when researchers started using cultured skin fibroblasts from these patients for experimentation. Increased incorporation of radioactive sulfate in the GAGs (Matalon and Dorfman, 1969) and accumulation of metachromatic granules in cultured skin fibroblasts from such patients (Danes and Bearn, 1965) were shown for the first time. Further studies showed that the decay of radioactive sulfated polysaccharides in the Hurler and Hunter fibroblasts were much slower than in normal fibroblasts suggesting faulty degradation (Fratantoni et al., 1968b). Subsequently it was shown that the mixing of either fibroblasts or culture medium from different MPS types namely Hurler with Hunter, can 'correct' the defect, normalizing the radioactive sulfate uptake and the decay of the newly sulfated GAGs. These observations and similar studies suggested that the defect in each of the MPS type was unique (Barton and Neufeld, 1972). With time it was shown that MPS could be grouped into complementation classes based on their ability to 'cross correct' in vitro and that the corrective factors could be shown to be a single degradative enzyme, the absence of which led to MPS (Neufeld and Cantz, 1971, Neufeld et al., 1975). Pioneering studies by different groups led to the
discovery of specific enzyme deficiency for each of the known MPS types (Bach et al., 1972; Dorfman and Matalon, 1972, 1976; Sly et al., 1972; Neufeld, 1983).

In the 70s and 80s most of the enzymatic steps involved in the degradation of the GAGs were described and purification of most MPS enzymes reported. Genes encoding most of the GAG degradative enzymes were cloned and characterized in the 90s. Elucidation of each normal DNA structure has been followed by a profusion of studies identifying mutations. Mutations underlying any one MPS are very numerous, but one or a few mutations may sometimes predominate in particular geographic or ethnic population (Scott et al., 1995).

2.2 MUCOPOLYSACCHARIDOSIS I (Clinical features)

Mucopolysaccharidosis type I (MPS I; MIM 252800) is the first described disorder of glycosaminoglycan metabolism (Hurler, 1919). It is an autosomal recessive, lysosomal storage disorder, caused by the deficiency or total absence of the enzyme α-L-iduronidase. This enzyme is required for the hydrolysis of nonreducing terminal α-L-iduronide glycosidic bonds in dermatan sulfate and heparan sulfate (Roden, 1980; Hopwood, 1989). Deficiency of this enzyme leads to accumulation of partially degraded or undegraded GAGs inside the lysosomes, the excess being excreted in the urine (Neufeld and Muenzer, 2001). This accumulation eventually results in cell, tissue and organ dysfunction leading to progressive disability in the patient which include differing degrees of involvement of connective tissue,
central nervous system, bony skeleton and heart (Brooks, 1993: DiNatale et al., 1993).

Originally MPS I was classified into three subtypes based on their clinical manifestations, Hurler (severe), Scheie (mild) and Hurler-Scheie (intermediate) syndromes (McKusick et al., 1972). The classification of the patients within these subtypes has been valuable to emphasize the clinical difference seen within MPS I. However, unusual clinical phenotypes that do not fit into these subtypes have been reported (Roubicek et al., 1985). It is now known that there is a continuous spectrum of clinical involvements observed within MPS I making it difficult to delineate them into 3 subtypes (Scott et al., 1995, Neufeld and Muenzer, 2001). Also biochemically, it is extremely difficult to distinguish between the subtypes and to date there is no satisfactory method for predicting the severity of the clinical phenotypes, because all the patients have the following features (Neufeld and Muenzer, 2001).

i) Excessive urinary excretion of DS and HS

ii) Deficiency or absence of IDUA activity even in the mildest form.

However for convenience, the assignment of the subtypes is made on the basis of clinical features.

2.2.1 Mucopolysaccharidosis I H (Hurler syndrome)

Hurler syndrome, though recognized as the prototype of all MPS, is not the representative of all MPS. It represents only the severe end of the
clinical spectrum for MPS I. It is a progressive disorder with multiple organ and tissue involvement that leads to death in childhood, usually by 10 years. A Hurler infant appears normal at birth but may have inguinal or umbilical hernias. The diagnosis is usually made between 4 and 18 months of age. A combination of skeletal deformities, recurrent ear and nose infections, inguinal and umbilical hernias and course facial features prompt medical attention (Cleary and Wraith, 1995). Children with Hurler syndrome are usually large in infancy but a deceleration of growth commonly occurs between 6 and 18 months. The developmental delay is usually apparent by 12 to 24 months, with a maximum functional age obtainable of 2 to 4 years followed by progressive deterioration. The developmental delay, chronic hearing loss and enlarged tongue leads to limited language skills in the patients. Most of the Hurler patients have been observed with ear infections, noisy breathing and persistent copious nasal discharge. The cornea begins to show progressive clouding during first year of life and glaucoma may occur in some patients with Hurler syndrome.

The hydrocephalus in Hurler syndrome is usually associated with increased intracranial pressure. Obstructive airway disease, respiratory infection and cardiac complications are the usual cause of death. The skeletal abnormalities in Hurler syndrome are described as dysostosis multiplex. These changes include large skull with thickened calvarium, premature closure of lamboid and sagittal sutures, shallow orbits, enlarged “J” shaped sella and abnormal spacing of teeth. The vertebral bodies are ovoid in the lower thorax and upper lumbar on their lower anterior margins, while their
upper portions remain hypoplastic, resulting in the gibbus deformity commonly seen in these patients (McKusick, et al., 1972).

2.2.2 Mucopolysaccharidosis I S (Scheie syndrome)

The Scheie syndrome patients show the mildest phenotype among the MPS type I. Initially this syndrome was classified as a separate entity under MPS V (Scheie et al., 1962). When the defective enzyme was shown to be á-L-iduronidase (Bach et al., 1972), this group was reclassified as MPS I S, an allelic variant of Hurler syndrome.

Scheie syndrome patients are characterized by normal intelligence, mild facial coarsening, joint stiffness, hepatosplenomegaly and corneal clouding. Radiological examination of Scheie patients shows minimal dysostosis multiplex, without the vertebral changes or the gibbus deformity seen in Hurler syndrome. There is coxa valga and slight radial and ulnar obliquity with V formation of their articular surfaces. Spatulate ribs, joint contractures and tapering of the proximal ends of metacarpals are often observed in these patients (Stevenson et al., 1976). Obstructive airway disease causing sleep apnea develops in some patients. Aortic valvular with stenosis and / or regurgitation occurs due to build up of GAG deposits on valves and chordae tendineae. Compression of the cervical cord by thickened dura, pachymeningitis cervicalis, with resulting myopathy can occur in MPS IS, though less commonly than in MPS I H/S. Deafness has been reported in some patients, but the etiology is unknown. The onset of significant
symptoms is usually after the age of 5 years, with the diagnosis commonly made between 10 and 20 years of age (Neufeld and Muenzer, 2001).

2.2.3 Mucopolysaccharidosis I H / S

(Hurier – Scheie syndrome)

The clinical phenotype of Hurler–Scheie syndrome is intermediate between the Hurler and Scheie syndromes. The onset of symptoms is usually observed between 3 and 8 years and survival to adulthood is common. This syndrome is characterized by progressive somatic involvement, including dyostosis multiplex with little or no intellectual dysfunction.

The radiological findings of the Hurler–Scheie compound patients show enlarged sella turcica, spatulate ribs, hypoplasia of the pelvic bones and femoral heads, flaring of iliac bone, coxa valga and minimal abnormalities of the vertebral bodies and metacarpals (Winters et al., 1976; Kaibara et al., 1983). Corneal clouding, joint stiffness, deafness and valvular heart disease can develop by early to mid teens and cause significant impairment and loss of functions. Some patients with MPS I H/ S have micrognathism that creates a characteristic facies. Pachymenigitis cervicalis and compression of the cervical cord due to mucopolysaccharide accumulation in the dura, occurs in MPS I H/S. Cardiac involvement and upper airway obstruction lead to mortality (Neufeld and Muenzer, 2001).
2.3 DEGRADATION OF GLYCOSAMINOGLYCANS

The enzyme deficiencies in the MPS have been elucidated after the understanding of normal pathways of glycosaminoglycan catabolism. The role of many of the enzymes became apparent only through the consequences of their absence. The glycosaminoglycans themselves are lysosomal degradation products derived by proteolytic removal of the protein core of proteoglycans (Neufeld and Muenzer, 2001).

2.3.1 Degradation of Heparan Sulfate

Heparan sulfate (HS) consists of glucuronic acid and L-iduronic acid residues, some of which are sulfated, alternating with α-linked glucosamine residues (Roden, 1980; Hopwood, 1989). The glucosamines are either sulfated or acetylated on the 6 hydroxyl. HS Proteoglycans are first subjected to proteolysis in early endosomes to produce single HS chains, which are then degraded by substrate structure-specific endo-α-glucuronidase to oligosaccharides (Hopwood, 1989). These oligosaccharides are transported from acid endosomes to the lysosomes where degradation proceeds from the non-reducing end by the sequential action of three glycosidases, five sulfatases and a biosynthetic enzyme that transfers an acetyl group from acetyl CoA to N-acetylglucosamine. The degradative pathway is shown in fig. 1.

The enzyme iduronate-2-sulfatase hydrolyses terminal iduronate-2-sulfate esters in HS. Alpha-L-iduronidase acts on the oligosaccharide product of iduronate -2-sulfatase to produce free iduronic acid and the substrate for
the next enzyme glucosamine-6-sulfatase. Sulphaminidase acts on the oligosaccharide product of glucosamine-6-sulfatase. Following de-N-sulphation of non-reducing end N-acetylglucosamine(GlcN) residues by sulphaminidase, a "biosynthetic" step is required to N-acetylate this N-acetylglucosamine product before degradation can proceed. This reaction involves the transfer of the acetyl group from cytoplasmic substrate acetyl-Co-A to the lysosomal substrate. This unusual enzyme activity, which forms bonds in the overall bond-breaking pathway, has its counterpart in the N-deacetylase involved in the heparan sulfate biosynthetic pathway in the Golgi (Lindahl et al., 1994). The next enzyme to act on the acetylated oligosaccharide product of the N-acetyltransferase is α-N-acetylglucosaminidase. Glucuronate-2-sulphatase is required to desulfate glucuronate-2-sulfate. The enzyme involved in the last step in the catabolism of HS is α-D-glucuronidase which hydrolyses the α-D-glucuronic acid residue.

Heparanase, an endoglucuronidase, has been identified in the catabolism of HS which cleaves the polymer into smaller fragments, some of which are released in the urine. This may provide an alternate pathway that limits the amount of GAGs stored in the tissues of patients with MPS I, II, III or VII (Neufeld and Muenzer, 2001).

### 2.3.2 Degradation of Dermatan Sulfate

Dermatan sulfate (DS) consists of iduronic acid and glucuronic acid residues alternating with α linked N-acetyl galactosamine residues (Roden,
Degradation takes place from the nonreducing end by the sequential action of three glycosidases and two sulfatases. The degradative pathway is shown in fig. 2.

The degradation begins with the action of iduronate-2-sulfatase which desulfates the C-2 sulfated iduronic acid at the non-reducing terminus of DS. The terminal α-L-iduronide is hydrolysed by α-L-iduronidase, producing a product with terminal N-acetylgalactosamine-4-sulfate. The desulfation reaction is catalyzed by the corresponding sulfatase enzyme and the terminal N-acetylgalactosaminide produced is acted upon by α-hexosaminidase isoenzymes A, B or S. α-D-glucuronidase is the final enzyme required to degrade DS.

An alternative pathway for DS degradation may exist wherein α-hexosaminidase A or S is able to hydrolyze the α-linked N-acetylgalactosaminide-4-sulfate residue to produce the monosaccharide N-acetylgalactosaminide-4-sulfate which is then hydrolysed by 4 sulfatase to inorganic sulfate and N-acetylgalactosamine. The precise contribution of these alternative reactions to the normal degradation of DS is unknown (Hopwood and Morris, 1990).

The enzymes described above are exoglycosidases and exosulfatases, that hydrolyse linkages only at the nonreducing terminus of the glycosaminoglycans. On the other hand hyaluronidase, an endohexosaminidase can cleave dermatan sulfate internally between N-acetylgalactosamine and the occasional adjoining glucuronic acid residue. Though the fragments of dermatan sulfate found in the urine of MPS patients
are probably generated by hyaluronidase, the role of this enzyme in the normal lysosomal degradation of DS is not known. Cleavage by hyaluronidase followed by excretion of the resulting fragments may be considered an alternative catabolic route and it appears to be limited to certain tissues, such as liver and does not occur in fibroblasts (Neufeld and Muenzer, 2001).

2.4 α-L-IDURONIDASE

The lysosomal enzyme α-L-Iduronidase (IDUA; EC 3.2.1.76), the enzyme deficient in MPS I, is one of the enzyme involved in the degradation of DS and HS. Its activity has been demonstrated in a variety of tissues such as human liver (Clements et al., 1985a,b), kidney (Rome et al., 1978), urine (Shapiro et al., 1976), lung (Schuchman et al., 1984a,b), fibroblasts (Hall and Neufeld, 1973; Hopwood et al., 1979), leukocyte (Liem and Hooghwinkel, 1975; Hopwood et al., 1979), cultured amniotic fluid cells (Hall and Neufeld, 1973), brain, spleen (Constantopoulos et al., 1976) and chorionic villi (Young, 1992).

The enzyme has been purified and characterized from human (Rome et al., 1978) and animal tissues (Stolzfus et al., 1992). It is a glycoprotein and contains 6 potential N-glycosylation sites (Kakkis et al., 1994). Enzymes purified from each tissue source differ in both native protein and subunit molecular mass. Furthermore, different catalytic properties have been reported for various preparations of purified enzyme. The differences may be due to the tissue source, the method of purification, the presence of
modifications or different assay protocols, which have made the comparison of kinetic data between the laboratories difficult.

Studies on IDUA biosynthesis in cultured skin fibroblasts using immunoprecipitation of the radiolabelled enzyme have shown that the maturation of IDUA is extremely slow compared to other lysosomal hydrolases. The results indicate that the enzyme is made in a precursor form (81 KDa) which is processed through 76 and 70 KDa intermediates to a 69 KDa native polypeptide which has the mannose-6-phosphate marker for lysosomal targeting (Scott et al., 1991a). The mature and active enzyme exists in a monomeric form.

IDUA constitutes only 0.005 % of the total cell protein. Hence immuno histochemical detection of IDUA using either mono or poly clonal antibodies is limited (Ashton et al., 1992). The protein sequence deduced from the nucleotide sequences of cDNA encoding human (Scott et al., 1992a), canine (Stoltzfus et al., 1992) and murine (Russell et al., 1998) α-L-iduronidase have a high degree of homology and predict proteins of 653, 655, 634 amino acids respectively. Analysis of the carbohydrate residues of recombinant human IDUA secreted by over expressing CHO cells reveals that all 6 glycosylation sites on the enzyme are utilized but mannose-6-phosphate marker is carried only on sites 3 and 6 (Scott et al., 1991a).

The in vivo substrate for the enzyme appears to be the two polysaccharides-dermatan sulfate and heparan sulfate. However the commonly used substrates for in vitro assay are synthetic, using which a
variety of assay protocols have been developed which include colorimetric, fluorimetric and radioactive methods. Also mono and polyclonal antibodies have been developed for the immunoassay of the enzyme.

In the colorimetric assay, estimation of phenol liberated upon hydrolysis of phenyl-α-iduronide represents the IDUA activity. However it has not been possible to demonstrate residual IDUA activity in MPS I patients using this substrate. Also synthesis of the substrate and assay procedure are cumbersome and less sensitive (Neufeld and Muenzer, 2001). Hence this methods has been replaced by radioactive and fluorimetric methods.

Radiolabelled substrates like iduronosyl-[1-3H]-anhydro-mannitol, iduronosyl-[1-3H]-anhydro-mannitol-6-sulfate and iduronosyl-[1-3H]-anhydrotalitol-4-sulfate that closely match the structure of the presumed natural substrates HS and DS have been developed (DiNatale et al., 1977; Hopwood and Muller 1979;1982; Muller and Hopwood, 1984). In the assay, the products formed on hydrolysis of the substrate are separated either by column chromatography or high voltage electrophoresis and then counted. Though less convenient, these 3 substrates have the ability to detect residual enzyme activity in MPS I fibroblasts.

In the fluorimetric assay 4-methylumbelliferyl-α-L-iduronide is used as the substrate. Hydrolysis of the substrate by IDUA releases 4-methylumbelliferone (4MU) which strongly fluoresces in alkaline medium against the substrate which exhibits only weak pH dependent fluorescence
The fluorescence is measured at an excitation wavelength of 360 nm and emission wavelength of 415 nm (Shapira et al., 1989). This is a sensitive, convenient and superior substrate compared to phenyl α-L-iduronide. It allows discrimination between homozygotes and heterozygotes for IDUA deficiency and has gained wider acceptance for the diagnosis of MPS I (Hopwood et al., 1979; Minami et al., 1980).

### 2.5 LOCALIZATION OF α-L-IDURONIDASE GENE (IDUA)

Initially the α-L-iduronidase gene locus was assigned to the chromosome 22 pter- q11 based on somatic cell hybrid studies (Schuchman et al., 1984c). Later in the year 1989, α-L-iduronidase was purified from human liver and sequenced (Clements et al., 1989). This helped in the isolation of a cDNA and genomic DNA clone of IDUA gene. Using this in situ hybridization studies the earlier chromosomal assignment was disproved and the IDUA gene was mapped to chromosome 4p16.3, which is further confirmed by Southern blot analysis of mouse-human cell hybrids (Scott et al., 1990). Further refinement localized it to D4S111, which is 1.5-2 megabases from Huntington disease gene and 600 kb from the telomere. (MacDonald et al., 1991). Several other genes also have been mapped to this region (Gandelman et al., 1992; Shiang et al., 1994), but it is unlikely that α-L-iduronidase plays a part in the expression of these disease phenotype.

#### 2.5.1 The gene structure

The human IDUA gene spans approximately 19 kb in length and is split into 14 exons. The first 2 exons are separated by an intron of 566 bp, a
large intron of about 13 kb follows and the remaining 12 exons are clustered within 4.5 kb. The 13 kb intron 2 contains an Alu repetitive element and a highly polymorphic VNTR repeat (Scott et al., 1991b; 1992c). All intron-exon splice junctions follow the GT/AG rule except for intron 11, which follows the GC/AG exception.

The potential promoter for IDUA gene has only GC box-type consensus sequences and possibly multiple transcription start sites consistent with a housekeeping promoter with low levels of transcription. The promoter is bounded by an Alu repeat sequence at the 5' end (Scott et al., 1992c). The direction of transcription is towards the centromere (Scott et al., 1992a) and transcription produces a 2.3 Kb mRNA. No consensus polyadenylation signal is found in the transcript and alternate splicing events occur at low levels (Bach et al., 1993; Scott et al., 1993a; Bunge et al., 1994) which may be of some functional significance, not necessarily associated with IDUA function (Scott et al., 1995).

The cDNA sequence analysis has shown an open reading frame of 1959 bp which encodes a 653 amino acid peptide (Scott et al., 1991a). The peptide consists of a 26 amino acid signal sequence at the amino terminus and contains 6 potential N-glycosylation sites (Scott et al., 1995). The canine and murine IDUA gene, cDNA and protein have been studied, all of which show a high percentage of homology to the human counterpart (Menon et al., 1992; Stoltzfus et al., 1992; Clarke et al., 1994a).
2.5.2 Mutations Identified

Mutations in this gene were first reported in 1992 (Scott et al., 1992; 1992d). According to Human Gene Mutation Database (HGMD 2004) till date 89 types of mutations have been reported in this gene (table - 4). Most of the mutations are clustered towards the beginning and middle of the gene (Fig.-3).

2.5.2.1 Chain termination mutation

The mutation W 402 X has been identified as a common mutation in Caucasian populations. It is present in Hurler patients who are homozygotes and MPS I patients including Scheie, who are compound heterozygotes (Moskowitz et al., 1993b). This mutation is associated with very severe clinical phenotypes in homozygotes. This leads to a G → A transition at nucleotide 1293 which alters the Trp 402 codon (TGG) to a stop codon (TAG). The translation gets terminated approximately 2/3 of the way through the 653 aminoacid protein. This mutation introduces a Mae I restriction endonuclease site into the gene, enabling detection of the mutation (Scott et al., 1992b).
The nonsense mutation, Q 70 X creates a C \rightarrow T transition at nucleotide 296 which alters a glutamine codon at position 70 (CAG) to a stop codon (TAG). The termination of translation occurs soon after the mature 74 KD aminoterminus of the IDUA protein. In homozygotes it is associated with an extremely severe clinical phenotype whereas compound heterozygotes show a wide range of clinical phenotypes (Scott et al., 1992a). Similar effects were produced by an alteration of the proline at position 583 to an arginine (Scott et al., 1992a).

2.5.2.2 Splice site mutations

The mutation 678-7 g \rightarrow a was identified in compound heterozygotes (Moskowitz et al., 1993b) and was responsible for Scheie phenotype. This mutation created a new acceptor splice site, causing 5 intronic nucleotides to be inserted into mRNA. This out of frame insertion led to an almost immediate termination codon. Since the normal splice site was not obliterated by the intron 5 mutation, it allows the normal synthesis of some completely normal enzyme. This finding demonstrates that just one allele, if it permits residual enzyme activity, can protect from severe disease (Neufeld 1991).

The splice site mutation, 678 - 7 g \rightarrow a alters the 3' consensus splice site of intron 5 and gives a mild phenotype (Moskowitz et al., 1993b). It is thought that the mild phenotype is due to the production of less than 1% of normally spliced mRNA and a correspondingly low amount of normal IDUA protein.
Another 3' splice site mutation 474-2 a → g is relatively a common allele and results in exon 4 skipping and a severe phenotype (Clarke et al., 1994b). Also the mutations 703del22ins10, 682 ins AC and Q310X affect m RNA splicing. All the three mutations use the -28 position of intron 5 as a cryptic 3' splice site. A low level of alternative splicing is also seen at this position in normal IDUA alleles (Bunge et al., 1994).

2.5.2.3 Insertion / Deletion mutations

A total of 24 insertions and deletions have been described in this gene. Out of 24, sixteen are small deletions ranging from 1bp to 12bp and six small insertions ranging from 1bp to 12 bp, one small indel and one gross indel (Human Gene Mutation Database 2004). In addition to this, a complex rearrangement with deletion of 22 bp followed by duplication of 10 bp has been described (Moskowitz et al., 1993a). Nine of these 14 mutations involve perfect or imperfect repeat sequence. Three of these mutations, 396 ins AC, 703del22ins10 and 682 ins AC have been shown to affect RNA splicing and / or stability. The mutations 134del12 974ins12, 1132del6 1277ins9 and Δ D445 do not induce frameshifts, only 974ins12 and Δ D445 results in mild phenotypes. Mutation 113del6 overlaps with a conservative missense mutation D349N and results in a severe phenotype. Also the 1342del6 mutation results in deletion of codons 16 - 19 in the signal peptide of IDUA protein. The patient homozygous for this mutation have a severe phenotype.

2.5.3 Population Studies

2.5.3.1 Druze and Arabs
Four mutant alleles are characterized in 7 families of Druze and Muslim Arab patients in Israel. 5 of them Druze patients (Bach et al., 1993). One allele has two amino acid substitutions, Gly to Arg in codon 409 of exon 9 and termination codon to Cys in exon 14. The other three alleles have mutations in exon 2 (Try 64 to termination), exon 7 (Gln 310 to termination) and exon 8 (Thr 366 to Pro). Transfection of mutagenized cDNA into cos-1 cells showed that the missense mutation Thr 366 to Pro permitted the expression of only trace amount of alpha L iduronidase activity. The Try 64 to termination mutation was accompanied by a very low level of mRNA and skipping of exon 2. Utilization of a cryptic splice site was observed with the Gln 310 to termination. Expression of Gly 409 to Arg mutagenized cDNA showed a reduction of less than half the enzyme activity whereas termination codon to Gly reduced the enzyme activity by 98% compared with expression of normal cDNA (Bach et al., 1993).

A heterozygous mutation in codon 492 of IDUA gene corresponding to a change of arginine to proline was reported (Tieu et al., 1995). This mutation created an Apa I site. No IDUA activity was observed when the cDNA was mutagenized and was expressed. Even though no activity was observed, this mutation must be presumed responsible for the mild Scheie phenotype.

2.5.3.2 Asian - Indian

A Hurler - Scheie patient of Asian Indian origin was reported to have a T to C transition in codon 490, converting leucine (CTG) to proline
(CCG) and creating a Sma I site (Tieu et al., 1994). No IDUA activity was detected when cDNA containing the L 490 P mutation was expressed in cos -1 cells. There was no evidence for heterozygosity either in the genomic sequence or in the restriction digest, suggesting that the mutation was present in homozygous form. However hemizygosity because of either deletion of the IDUA gene on one chromosome or uniparental disomy had not been ruled out. The parents were not known to be consanguineous. Homozygosity had been observed previously only in consanguineous families or for the most common mutations, W402X and Q70X. It is therefore possible that the L 490P mutation is relatively common among Indian MPS I patients (Tieu et al., 1994; 1995).

2.5.3.3 Japanese

Two common mutations, 704 ins 5 (18%) and R89Q(24%) in a group of 19 Japanese patients were reported (Yamagishi et al., 1996). The novel 5 bp insertion between the thymidine at 704 and a cystosine at 705, is seen only in the Japanese population. The other missense mutation R89Q is seen also in Caucasians, although uncommonly. No Japanese patient was found to carry the W402X or Q70X alleles, the two most common mutations in Caucasians. Homozygosity for the 704 ins 5 mutation is associated with a severe phenotype and for the R89Q mutation with mild phenotype. Compound heterozygosity for these two mutations produced an intermediate phenotype (Yamagishi et al., 1996).
2.5.3.4 Others

Homozygosity for a Thr 364 to Met mutation in the IDUA gene of a Chinese patient with Hurler-Scheie syndrome was identified (Lee-Chen and Wang 1997). Similarly, compound heterozygosity for a maternal allele with a Leu 346 to Arg and a paternal allele with a C to G transversion at position 3 of the 3' splice site of intron 2 were identified (Teng et al., 2000). In transfected cos 7 cells L346R showed no appreciable IDUA activity, although it did not cause an apparent reduction in IDUA mRNA or protein level. The splice site mutation profoundly affected normal splicing, leading to a very unstable mRNA. Expression of IDUA cDNA containing the mutated acceptor splice site showed trace amounts of enzyme activity. The results provided further support for the importance of cystine at the position 3 in RNA processing.

Although Hurler and Hunter syndromes are recognized as "prototype" MPS diseases, they are relatively rare disorders, with incidence estimated as 1/50,000 and 1/100,000 births (Hopwood and Morris 1990; Lowry et al., 1990) respectively. Thus the a priori probability that both disorders would occur in the same individual is about 1 in 5 billion. Only one such patient has been reported (Aronovich et al., 1996). The biochemical tests indicated the concomitant presentation of both disorders. The analysis of the IDUA gene and IDS gene (MPS II) showed the presence of the common IDS gene mutation R468W and the common IDUA mutation W402X in the patient. Notably, a new IDUA gene mutation A300T was also
identified. This A300T mutation is the first IDUA pseudodeficiency gene to be elucidated at the molecular level (Aronovich et al., 1996).

2.5.4 Mutation frequencies in different populations

2.5.4.1 Brazilian population

Twenty four MPS I patients from different regions of Brazil have been reported (Matte et al., 2000). The Q70X mutation was present in only one patient whose other allele was A327P. This was the first time this genotype was described. The reported frequencies for Q70X and W 402 X are markedly higher in patients of Anglo-Saxon origin (Scott et al., 1992b). In Italian patients frequencies are of 13% for Q70X and 11% for W402X (Gatti et al., 1997). In Spanish patients the frequency of 10% for P533R, 10% for Q70X and 60% for W402X are reported. The frequency of W402X (20.6%) mutation in Brazilian population is slightly higher than the frequency found among Italian patients (11%) and much lower than the Spanish frequency (60%).

2.5.4.2 European population

The mutations W402X (37%) and Q70X (35%) are reported to be more common among European MPS I patients (Bunge et al., 1994). In addition to W402X and Q70X, L218P is found to be the third frequent mutation (6.5%). The mutation W402 X is more frequent (48%) in West and Central European countries (mainly Netherlands and Germany) and less common (17%) in Northern Europe (Norway and Finland). Founder effects or
genetic drift may play a role for the uneven distribution of these mutations (Bunge et al., 1994).

2.5.4.3 Moroccan population

MPS I patients of Moroccan descendent were screened for mutations. The mutation P533R was found in 92% of the alleles (Alif et al., 1997). This is the highest frequency of this mutation detected in Hurler syndrome. This mutation has been identified in 3% of the mutant alleles in Europeans (Scott et al., 1992b) and in 11% of mutants in Italians (Gatti et al., 1997). The frequent occurrence of the P533R mutation in homozygous form probably results from the frequency of consanguineous union. (Alif et al., 1997).

2.5.4.4 Former Soviet Union (FSU)

The genotype of 25 unrelated MPS I patients were studied from the FSU (Voskoboeva et al., 1998). The mutations W402X and Q70X were found to be common with the frequency of 4 and 44% respectively. The frequency of the mutations in patients from Scandinavia are 17% for W402X and 62% for Q70X, while patients from western and central European countries W402X and Q70X accounts for 48 and 19% respectively (Scott et al., 1995). The frequency of W402X and Q70X alleles is somewhat similar to the Scandinavian population than between the FSU and central Europe. It is most likely that the W402X alleles is of Anglo - Saxon or Celtic origin (Voskoboeva et al., 1998).
2.5.5 Polymorphisms and nonpathogenic sequence variations in the gene

Thirty polymorphisms and/or nonpathogenic sequence variations have been reported (Scott et al., 1995; www.snp.cshl.org, 2005). Of this, 18 are exonic variations and 12 are intronic variations. This is an extraordinarily large number of polymorphisms compared to many other disease genes of similar size (Hopwood et al., 1993). Of the 30 nonpathogenic sequence variants, only one which was detected in an Iranian patient, involves an insertion of a C in a string of nine Cs in intron 8. Of the remaining 29 single base changes, 15 occur in CpG dinucleotides, 6 of the nonpathogenic amino acid changes are in residues that are not conserved between the canine and/or murine and/or human IDUA, and the remaining one (G409R) is in an area of generally lower homology (Scott et al., 1995).

The effect of these polymorphisms and/or nonpathogenic sequence variation on the stability and processing of the IDUA mRNA, the rate of translation of the IDUA protein, the activity, of the enzyme stability and transport of the IDUA protein are not known. However, these polymorphisms may be a contributing factor to variation in IDUA activity in healthy individuals. Also these nonpathogenic sequence variations may be responsible for some of the mild MPS I allele in combination with a severe allele (Scott et al., 1995). It is hypothesized that the polymorphism A361T may play a major role in modulating the effect of the causative mutations (R89Q) in these patients, thus altering the clinical phenotype (Scott et al.,
This idea is not entirely unique, since an intronic polymorphism in the CFTR gene has been shown to alter disease expression in cystic fibrosis.

2.5.6 Genotype - Phenotype correlation

The continuous spectrum of clinical phenotype ranging from near normal to severe debilitating forms in MPS I is caused by the combination of different mutant alleles at the IDUA locus (Hopwood and Morris 1990). The genotype to phenotype correlations for MPS I can be considered most promising. In general, any combination of two severe alleles leads to the severe form of MPS I. The mutation 396inAC, which is expected to produce a frameshift mutation leading to premature termination of transcription and thus a severe phenotype, gives an intermediate phenotype in compound heterozygosity with the severe Q70X allele (Bunge et al., 1995). This is an exception to the consistent genotype to phenotype correlation in MPS I. But the intermediate and mild forms can occur in the presence of one severe allele and one mild allele (Scott et al., 1995). On the contrary, a difference in the clinical phenotypes was observed in sibs of same genotype (Scott et al., 1993b). In addition, phenotype descriptions available are often drawn from many different physicians with no attempt at standardization making it difficult to compare phenotypes (Scott et al., 1995).

The IDUA gene overlaps with the sat-1 gene which is involved in the sodium independent transport of sulfate in a tissue specific manner. Therefore a mutation in the IDUA gene may affect the expression of sat-1 gene, altering the MPS I phenotype in an indirect way. Hence always caution
should be exercised in prediction, particularly for novel combination of alleles (Scott et al., 1995).

2.6 PRENATAL DIAGNOSIS

Mucopolysaccharidoses as other genetic disorders can be diagnosed in utero and the birth of affected children can thus be terminated in pregnancy (Dorfman and Matalon 1972). Prenatal detection of MPS I was reported first using $^{35}$S - sulphate incorporation test in cultured amniotic fluid (Fratantoni et al., 1968a). Later it has been achieved by using phenyl $\alpha$-L-iduronide as the substrate to examine the level of $\alpha$-L-iduronidase activity in cultured amniotic fluid cells derived from parents of such patients (Hall and Neufeld 1973). Various tissues like amniotic fluid, amniotic fluid cells, chorionic villi etc, have been used for the diagnosis. The amniotic fluid can be drawn at the second trimester and used for detection of accumulated DS and HS (Mossman and Patrick 1982). The amniotic fluid cells obtained at 18 weeks of gestation are grown in culture and used for the diagnosis of MPS I (Gatti et al., 1985). The diagnosis by direct assay of $\alpha$-L-iduronidase in chorionic villi using 4-methylumbelliferyl $\alpha$-L-iduronide as a substrate has been possible and highly accurate, even though $\alpha$-L-iduronidase activity was considerably lower than in cultured cells (Young 1992).

The activity of $\alpha$-L-iduronidase in the leukocytes of normal individuals, patients and heterozygous carriers were studied (Tsvetkova et al., 1991). The assays were carried out using 4- methylumbelliferyl-$\alpha$-L-iduronide and 4-trifluoromethyl umbelliferyl-$\alpha$-L-iduronide as substrates.
The studies showed that the latter can serve as a specific substrate for \( \alpha \)-L-iduronidase and can thus be used to demonstrate the enzyme deficiencies in patients and decreased enzyme activity in heterozygous carries. When used for prenatal diagnosis, in amniotic fluid cell cultures, the substrate proved superior since the enzyme deficiency could be detected directly in pieces of tissue and in placenta owing to the bright yellow fluorescence emitted by the product.

The pseudodeficiency of \( \alpha \)-L-iduronidase has been reported (Gatti et al., 1985, Whitley et al., 1987; Taylor and Thomas 1994; Aronovich et al., 1996). So the prenatal diagnostic studies would need to be interpreted with caution in cases of heterozygotes having a pseudodeficiency allele.

2.7 CARRIER DETECTION

Carrier detection for autosomal recessive mucopolysaccharidoses generally depends on measurement of the relevant enzyme in leukocytes, fibroblasts or serum. In the case of MPS I, it has been shown that the mean specific activities of skin fibroblast iduronidase in normal individuals and in carriers are clearly distinct, although overlap of high heterozygote values and low normal values occurred (Hall and Neufeld 1973). However, the overlap was more apparent for fibroblasts making leukocytes a better choice for carrier detection (Wenger and Williams 1991). Many workers have found the obligate heterozygotes have about 50% level of IDUA activity in leukocytes (Dulaney et al., 1976; Kelly and Taylor 1976; Omura et al., 1976; Momoi et al., 1977; Minami et al., 1980a; Priya et al., 1997; Mahalingam et al., 2002) when compared to normals.
DNA analysis will have immediate practical application in carrier detection, since it can provide unambiguous information on the carrier status. This can be done by testing for specific mutations or polymorphic markers.

2.8 ANIMAL MODELS

Animal models are available for most of the MPS. The available animal model for human MPS I include canine (Shull et al., 1982) and murine (Russel, et al., 1998). The biochemistry and the pathophysiology of the affected animals are very close to those in human patients. This facilitates testing new therapies such as bone marrow transplantation, enzyme replacement therapy and gene therapy.

2.8.1 Canine Model

The Canine IDUA gene has 14 exons and spreads over 13kb. The cDNA encoding α-L-iduronidase has been cloned and characterized (Stoltzfus et al., 1992). G to A transition in the donor splice site in intron I has been identified. This mutation causes retention of intron I in RNA and leads to premature termination at the exon - Intron Junction (Menon et al., 1992). Intravenous administration of purified, recombinant human α-L-iduronidase to homozygous affected animals improved the situation to a great extent. Light and electron microscopic examination showed normalization of lysosomal storage in liver, spleen and kidney glomeruli but there was no improvement in brain, heart valve or cornea (Shull et al., 1994).
2.8.2 Murine Model

The murine model permits evaluation of the pathophysiology of lysosomal storage disorders and provides a model for therapy protocols (Clarke et al., 1997, Russell et al., 1998). Murine MPS I models have been created by targeted disruption of the murine IDUA gene which results in severe form of MPS I. The affected mice showed a progressive clinical course with the development of coarse features, altered growth characteristics and a shortened life span. Progressive lysosomal accumulation was seen in all tissues (Clarke et al., 1997). The mouse IDUA gene has been localized to the proximal portion of chromosome 5 (Koizumi et al., 1992).

2.9 THERAPY

The observation that defective GAG catabolism in cultured cells was corrected by adding normal cells (Fratantoni et al., 1968b) paved the way for therapy to MPS. Presently therapies are available for MPS I patients. Bone marrow transplantation has some value in treating both somatic tissue and central nervous system (CNS) pathology, however this procedure carries significant risk (Braunlin et al., 2003). Enzyme replacement therapy (ERT) has recently become available for MPS I patients, but as intravenous infusion of enzyme is not effective in accessing sites of brain pathology, due to the blood-brain barrier, it is currently limited to the treatment of patients without CNS disease (Kakkis et al., 2001; Wraith, 2002). Studies on mutational analysis and phenotype-genotype correlation have reflected the overall conclusion that genotype is often not informative for predicting clinical severity and disease progression in MPS I patients. Therefore the ability to evaluate
patients using biochemical parameters will be important in deciding on therapeutic alternatives, such as BMT and/or ERT for MPS I and for monitoring patients receiving therapy.

2.9.1 Bone Marrow Transplantation

Bone marrow transplantation (BMT) has been performed for patients with MPS I since the early 1980s. BMT may be an effective treatment for MPS I, especially if it is performed before the onset of developmental decline. Clinical studies of BMT for MPS I patients demonstrated diminished hepatosplenomegaly, cardiomyopathy, coronary occlusion, airway obstruction, and neurological degeneration, but BMT did not improve the bone dysplasia heart valve deterioration, or corneal clouding. In these studies, up to 35% of patients died during and directly following BMT from treatment regiment-related toxicities, graft-versus-host disease, and disease manifestation (Peters, et al., 1996). BMT extends the life span of patients surviving the procedure, but the morbidity and mortality of allogeneic BMT and the need to identify a suitably matched marrow donor limit its usefulness (Kobayashi, et al., 2005).

2.9.2 Enzyme Replacement Therapy

Enzyme replacement therapy for lysosomal storage disorders depends on the efficient uptake of the enzyme into the tissue of patients. This uptake is mediated by oligosaccharide receptors including the cation independent mannose -6- phosphate receptor and the mannose receptor. Fusion of the human lysosomal
enzyme alpha-L-iduronidase with the receptor associated protein was efficiently endocytosed by lysosomal storage disorder patient fibroblasts. Uptake was specifically mediated by the low-density lipoprotein receptor. The advantage of the lipoprotein receptor over oligosaccharide receptor is the more efficient cellular delivery of the enzyme. (Prince, et al., 2004).

Initially, the lysosomal enzymes were purified from different tissues (Kakkis et al., 1994), for enzyme replacement therapy which was found to give only transient relief and also the enzymes lacked mannose-6-phosphate (Unger et al., 1994), the lysosome targeting marker. Cloning and characterization of the cDNA produced active recombinant enzyme (Unger et al., 1994) carrying the mannose-6-phosphate.

Presently, enzyme replacement therapy has been demonstrated in animal models of mucopolysaccharide storage disorders. The administered enzyme reduced glycosaminoglycan storage in the models. Clinical studies of enzyme replacement therapy are currently underway for mucopolysaccharide storage disorders. The complexity and heterogeneity of the disorders provide significant challenges for clinical study design and evaluation (Kakkis et al., 2001).

Recombinant human alpha-L-iduronidase significantly reduced hepatomegaly and urinary glycosaminoglycan in severely affected patients. It significantly improved respiratory function and physical capacity of the patients (Wraith, et al., 2004). In general, enzyme replacement therapy (ERT) is of clear clinical benefit for nonneuronopathic subset of MPS I patients, though it remains financially costly and
requires ongoing therapy. On the other hand systemic ERT is not expected to be clinically beneficial for the neurological features of MPS I due to poor penetrance of the recombinant enzyme across the blood-brain barrier.

Laronidase (recombinant human alpha-L-iduronidase) enzyme replacement therapy has been developed as a treatment strategy for MPS I patients and has been approved for clinical trials (Wraith, et al., 2005). Recently, laronidase became commercially available as a long-term enzyme replacement therapy. Results from the phase I/II and III extended clinical studies have shown that laronidase alleviates many systemic signs and symptoms of the progressive multisystemic disease. Significant and sustained reductions in urinary glycosaminoglycan excretion and hepatomegaly have also been observed. (Wraith, et al. 2004; Wraith, et al. 2005; Wraith, 2005).

2.9.3 Gene Therapy

A recombinant adeno-associated virus vector (vTRCA1) transducing the human iduronidase gene was injected intravenously into one day old MPS I mice. High levels of IDUA activity were present in the plasma of the treated animals that persisted for the 5 month duration of the study. The treated animals with measurable plasma IDUA activity exhibited histopathological evidence of reduced lysosomal storage in a number of tissues and were normalized with respect to urinary GAG excretion. (Hartung, et al., 2004).
Human IDUA cDNA was transferred through lentiviral vector to MPS I fibroblasts. It was observed that the level of enzyme expression in transduced cells was 1.5 fold the level found in normal cells, the expression persisted for at least two months. (Di Natale, et al., 2002).

The MND-MFG alpha IDUA vector showed high level, long term expression of the transgene in both canine and human alpha IDUA deficient fibroblasts. The effectiveness of this vector for in utero gene transfer and expression in multiple tissues was assessed by injecting viral supernatant into MPS I fetuses and evaluating transduction efficiency and enzyme expression at various times after birth. (Meertens, et al., 2002)

2.9.4 Others

An alternative approach to somatic gene therapy is to deliver a therapeutic protein by implanting recombinant cells that are immunologically protected from graft rejection with alginate microcapsules. Madin-Darby canine kidney cells were genetically modified to express either human growth hormone or canine alpha iduronidase, then enclosed in alginate ply 1 lysine alginate microcapsules. The encapsulated cells were implanted into the brain under steoreotaxic guidance. The brains were monitored with computed tomographic scans before and after surgery and examined biochemically and histologically. Delivery of gene products as measured in the plasma and cerebrospinal fluid sampled periodically through 21
days or in various regions of the brain after death showed that the delivery of both gene products was extremely low but detectable. (Barsoum et al., 2003)